TITLE: Low pH in Feed Test Procedure

METHOD NO.

MATERIAL: Activate DA™

TEST: Anti-bacterial activity of organic acids measured in feed at low pH

SCOPE: Anti-bacterial activity of organic acids is measured in feed at low pH to simulate the low pH and moisture conditions in the upper digestive tract of animal.

MATERIALS:

1. Finished feed: mash or crumble, swine or poultry
2. Fresh culture of *Salmonella* and *Escherichia coli*
4. Brilliant Green Agar or other selective media for salmonella enumeration
5. MacConkey Agar or other selective media for e. coli enumeration
6. Incubator set at 40C for the assay, and 37C for bacteria enumeration (plating)
7. Pipettes and sterile tips
8. Sterile tubes (50 ml)
9. Hydrochloric acid

SAFETY CONSIDERATIONS:

1. Mouth pipetting is not allowed, automatic pipettes or pipette bulbs must be used.
2. Use appropriate gloves where necessary.
3. Dispose of all hazardous waste properly. Autoclave all wastes containing salmonella or e. coli.

PROCEDURE:

Prepare fresh cultures of salmonella and e. coli:

1. Grow a fresh culture of salmonella or e. coli overnight at 37C in Tryptic Soy Broth (or appropriate media for the particular strain of bacteria)
2. Determine the counts by direct plating
3. Keep the culture at 4C until use. Prepare fresh cultures every 2 weeks.

Determine the amount of HCL needed to bring the feed to pH 4.0

1. Prepare 150mM HCL solution from concentrated HCl (12.1N HCl),
2. Weight out 5g of mash or crumbled feed in 50ml tubes,
3. Add 150mM HCl and DI H2O at different proportions (see the table below) to achieve a total volume of 15 ml,

150mM HCl	7.25 ml	7.50ml	7.75 ml	8 ml	8.25ml
DI H2O	7.75 ml	7.50ml	7.25 ml	7 ml	6.75ml
Total volume	15 ml	15ml	15 ml	15 ml	15 ml

4. Vortex the samples for ~1 min, keep at 40C for ~20min (preferable with mixing) for the pH to equilibrate,

5. Adjust the ratio between HCl and H2O until the pH of the feed is at ~ 4.0 (A range of 3.8 to 4.0 is acceptable).

Set up the following treatments (in 50 ml sterile tubes):

	Treatments	Dose	Reps.	Feed	Inoculant (cfu/g of feed)
1	control		2-3	5 gram	40,000
2	Activate DA	0.3%	2-3	5 gram	40,000
3	Activate DA	0.5%	2-3	5 gram	40,000

1. Weigh out 5g of finished feed in a sterile 50 ml centrifuge tube.
2. Add Activate DA to treatments 2 and 3 (15mg in the 0.3% treatment, and 25mg in the 0.5% treatment).
3. Add HCl and DI H2O to bring the pH to 4.0 (pre-determined for each feed, see the procedures above),
4. Inoculate with Salmonella or E. coli to give a final concentration of 40,000 cfu per ml of sample (40,000 cfu/ml x 15 ml = 600,000 cfu/tube).
5. Incubate the samples for 90 minutes in a 40C incubator (preferably with mixing on an end to end rotator, but not required).
6. At the end of 90 minutes incubation, prepare 1:10 dilution of sample in sterile H2O (1ml sample and 9 ml H2O)
7. Plate the following samples on Brilliant Green agar (*salmonella*) and MacConkey agar (*E. coli*) and incubate plates at 37C overnight.
   100ul of 1:10 dilution from step 6
   100ul of undiluted sample
8. Count colonies the next day, determine cfu/ml sample, and compare with control.

ANALYTICAL TIME:

REFERENCE:
ATTACHMENTS : None

DOCUMENT CONTROL DATES :
Issue & Effective Date:
Prepared/Revised by: Date:
Approved by: Date: